Oncogene (2003) 22, 6609–6620

www.nature.com/onc

Small-molecule cyclin-dependent kinase modulators

Adrian M Senderowicz*,[1]

[1]Molecular Therapeutics Unit, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892-4330, USA

Aberrations in cell cycle progression occur in the majority of human malignancies. The main pathway affected is the retinoblastoma (Rb) pathway. The tumor suppressor gene Rb is an important component in the G_1/S transition and its function is abnormal in most human neoplasms. Loss in Rb function occurs by the hyperactivation of the cyclin-dependent kinases (cdk's). Therefore, modulation of cdk's may have an important use for the therapy and prevention of human neoplasms. Efforts to obtain small-molecule cdk modulators yielded two classes of modulators: direct and indirect modulators. Direct cdk modulators are small molecules that specifically target the ATP binding site of cdk's. Examples for this group include flavopiridol, roscovitine and BMS-387032. In contrast, indirect cdk modulators affect cdk function due to modulation of upstream pathways required for cdk activation. Some examples include perifosine, lovastatin, and UCN-01. The first example of a direct small-molecule cdk modulator tested in the clinic, flavopiridol, is a pan-cdk inhibitor that not only promotes cell cycle arrest but also halts transcriptional elongation, promotes apoptosis, induces differentiation, and has antiangiogenic properties. Clinical trials with this agent were performed with at least three different schedules of administration: 1-, 24- and 72-h infusions. The main toxicities for infusions $\geqslant$24-h are secretory diarrhea and proinflammatory syndrome. In addition, patients receiving shorter infusions have nausea/vomiting and neutropenia. A phase II trial of patients with advanced non-small-cell lung carcinoma using the 72-h infusion every 2 weeks was recently completed. The median overall survival for the 20 patients who received treatment was 7.5 months, a survival similar to that obtained in a randomized trial of four chemotherapy regimens containing platinum analogues in combination with taxanes or gemcitabine, or with gefitinib, a recently approved EGFR inhibitor for the treatment of advanced lung cancer. Based on these encouraging results, a phase III trial comparing standard combination chemotherapy versus combination chemotherapy plus flavopiridol is currently under investigation. The second example of direct small-molecule cdk modulator tested in clinical trials is UCN-01 (7-hydroxystaurosporine). UCN-01 has interesting preclinical features: it inhibits Ca^{2+}-dependent PKCs, promotes apoptosis, arrests cell cycle progression at G_1/S, and abrogates checkpoints upon DNA damage. The first phase I trial of UCN-01 demonstrated a very prolonged half-life. Based on this novel feature, UCN-01 is administered as a 72-h continuous infusion every 4 weeks (in second and subsequent cycles UCN-01 is administered as a 36-h infusion). Other shorter schedules (i.e. 3 h) are being tested. Dose-limiting toxicities include nausea/vomiting, hypoxemia, and insulin-resistant hyperglycemia. Combination trials with cisplatin and other DNA-damaging agents are being tested. Recently, phase I trials with two novel small-molecule cdk modulators, BMS 387032 and R-Roscovitine (CYC202), have commenced with good tolerability. In summary, novel small-molecule cdk modulators are being tested in the clinic with interesting results. Although these small molecules are directed towards a very prevalent cause of carcinogenesis, we need to test them in advanced clinical trials to determine the future of this class of agents for the prevention and therapy of human malignancies.
Oncogene (2003) **22,** 6609–6620. doi:10.1038/sj.onc.1206954

Keywords: cell cycle; molecular targets; cyclin-dependent kinases; flavopiridol; UCN-01; CYC202; BMS 387032; drug development

*Correspondence: AM Senderowicz, Molecular Therapeutics Unit, Oral and Pharyngeal Cancer Branch, National Institute of Craniofacial and Dental Research, National Institutes of Health, 30 Convent Drive, Building 30, Room 212, Bethesda, MD 20892-4330, USA; E-mail: sendero@helix.nih.gov

Brief overview of cell cycle regulation

The regulation of cell cycle and proliferation has been extensively studied in the last few years and a consensus paradigm of the regulation of the cell cycle regulation has been developed (Sherr, 1996; Morgan, 1997). According to this paradigm, the master switch of the cell cycle is the retinoblastoma (Rb) family of proteins. Proliferation occurs by the phosphorylation of these family of proteins by the cyclin-dependent kinases (cdk's) (Figure 1) (Sherr, 1996). There are at least nine cdk's (Sherr, 1996; Morgan, 1997). These kinases are activated by D-type cyclins (D1, D2, and D3) and cyclin E, and inhibited by two families of cdk inhibitors (CKI's), the Ink ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, and $p19^{Ink4d}$) and Cip/Kip families ($p21^{Cip1}$, $p27^{Kipl}$, and $p57^{Kip2}$) (Sherr, 1996; Morgan, 1997).

Rb proteins are pocket proteins that sequester E2F transcription factors preventing them from activating critical genes in cell proliferation (Wang *et al.*, 2001; Yu *et al.*, 2001). After Rb phosphorylation by cdk4 and/or cdk6 complexes during the G_1 phase and cdk2 at the

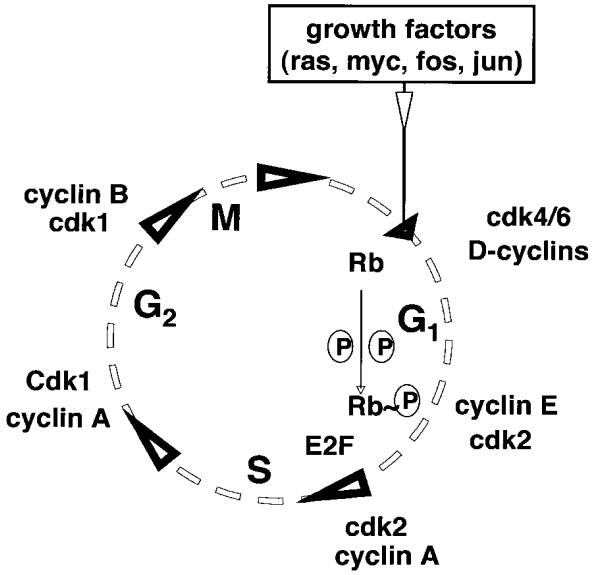

Figure 1 Schematic representation of cdk's and cell cycle control

G_1/S interphase, E2F proteins are released and promote the transcription of genes essential for transition to the S phase of the cell cycle (Lees *et al.*, 1993; DeGregori *et al.*, 1995). cdk4,6/D-type cyclins therefore execute their critical functions during the mid-to-late G1 phase, as cells cross a G_1 restriction point and become independent of mitogens for completion of the division cycle. These features suggest that the fundamental role of these complexes is to integrate extracellular signals with the cell cycle machinery (Sherr, 1996; Morgan, 1997).

Other important points of regulation have been described in G_2 and mitosis. In these phases also, the specific expression of certain regulators is essential to control the correct sequence of events that lead to cell division. Basically, the cyclins B1, B2, and its partner cdc2 (cdk1) together with other kinases and phosphatases (wee1, cdc25) regulated the final phases of the cell cycle (Figure 1)

In the last decade, several proteins that participate in the tight control of cell division have been found to be mutated, deleted, amplified, or overexpressed in human tumors. In the first part of this review, we summarize the principal points of deregulation found in human tumors with particular emphasis on lung cancer.

Cell cycle alterations in human neoplasms

In the last few years, it became clear that cyclins, cdk complexes, and other cell cycle regulators are mechanistically involved in the development of human tumors (Motokura and Arnold, 1993; Weinberg, 1996; Jacks and Weinberg, 1998; Robles *et al.*, 1998; Rodriguez-Puebla *et al.*, 1999). This is consistent with a large body of literature showing the importance of inactivation of the Rb pathway in tumor development (Hatakeyama and Weinberg, 1995; Weinberg, 1996; Senderowicz, 2002b). The inactivation of Rb can be produced by direct mutation of the Rb protein, but this is a relatively rare event occurring only in Rb's, osteosarcomas, and a minority of breast and some other tumors (Hunter and Pines, 1994; Sherr, 1996; Weinberg, 1996). More frequent alterations of this pathway occur by functional inactivation of Rb by hyperphosphorylation. This is normally the result of elevated cdk activities caused by overexpression of cyclins, cdk's.

For example, several laboratories have reported that some tumors show loss of Rb or, alternatively, overexpression of cyclin D1 (Bartek *et al.*, 1993; Bartkova *et al.*, 1995, 1996). Similarly, in other tumors, loss of $p16^{Ink4a}$ and Rb are mutually exclusive (Aagaard *et al.*, 1995; Lukas *et al.*, 1995a, b). This observation led to the hypothesis that inactivation of the cyclin D/CDK/p16/pRb pathway can promote tumor development and that either loss of the suppressor activity of Rb or $p16^{Ink4a}$, or overexpression of cyclin D1 can over-ride this checkpoint (Sherr, 1996; Weinberg, 1996). Several reports have implicated D-type cyclins in neoplastic development, although limited information is available on the participation of its partner, cdk4, in these events. The involvement of cdk4 in the neoplastic process was suggested by the fact that cdk4 amplification and/or overexpression were detected in human glioblastomas, but in these tumors overexpression and/or amplification of D-type cyclins were not detected (Sonoda *et al.*, 1995; Ichimura *et al.*, 1996). In addition, cdk4 mutations were identified in patients with familial melanoma (Wolfel *et al.*, 1995), and, recently, amplification and overexpression of cdk4 were also detected in sporadic breast carcinomas (An *et al.*, 1999), ovarian carcinomas (Masciullo *et al.*, 1997), and sarcomas (Kanoe *et al.*, 1998). Taken together, proteins that govern cell cycle control are reasonable targets for cancer therapy (Meijer, 2000; Senderowicz, 2002b).

Manipulation of cdk activity for therapeutic purposes

Several strategies could be considered to modulate cdk activity (see Figure 2). These strategies are divided into direct effects on the catalytic cdk subunit or indirect modulation of regulatory pathways that govern cdk activity(Senderowicz, 2000; Senderowicz and Sausville, 2000). Small molecular endogenous cdk inhibitors (SCDKI) are compounds that directly target the catalytic cdk subunit. Most of these compounds modulate cdk activity by interacting specifically with the ATP-binding site of cdk's (De Azevedo *et al.*, 1997; Meijer and Kim, 1997; Zaharevitz *et al.*, 1999; Senderowicz, 2000; Senderowicz and Sausville, 2000; Senderowicz, 2002b). Examples of this class include flavopiridol, roscovitine, aminothiazole, UCN-01 (7-hydroxystaurosporine), and alsterpaullone (See Figure 2). The second class are compounds that modulate cdk activity by targeting the regulatory upstream pathways that govern cdk activity: by altering the expression and synthesis of the cdk/cyclin subunits or the cdk inhibitory proteins; by modulating the phosphorylation of cdk's; by targeting cdk-activating

kinase (CAK), cdc25, and wee1/myt1; or by manipulating the proteolytic machinery that regulates the catabolism of cdk/cyclin complexes or their regulators (see Figure 2, panel b) (Senderowicz, 2000; Senderowicz and Sausville, 2000; Senderowicz, 2002a, b). Examples for this class of compounds include perifosine and UCN-01, among others.

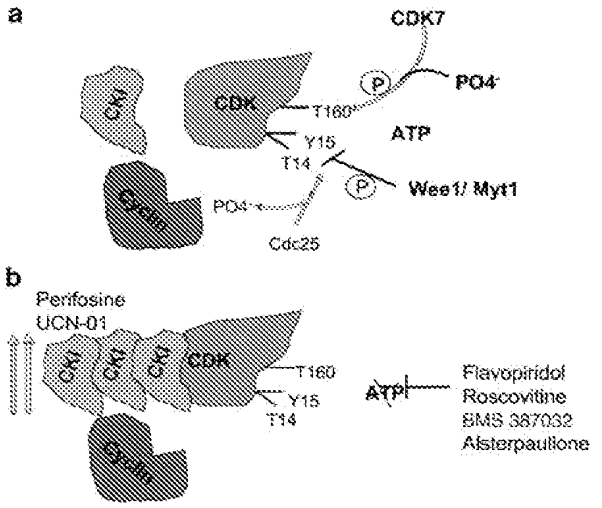

Figure 2 Modes of action for cell cycle modulators. There are two families of small-molecule cdk modulators, the direct and the indirect cdk modulators. Indirect modulators interact with upstream pathways, leading to loss in cdk activity. Loss in cdk function may occur due to loss in mass of catalytic subunit and/or cofactors, increased endogenous inhibitors, by increased wee1/myt1, or by loss in cdk7 or cdc25c activity. However, the most successful way of modulating cdk is by competing with ATP binding for cdks

Small-molecule cdk modulators (Tables 1 and 2)

As mentioned previously, cdk's can be modulated by direct effects on the catalytic subunit and/or by disruption of upstream regulatory pathways. Several examples and mechanisms are described in Tables 1 and 2 and elsewhere (Meijer, 2000; Senderowicz, 2000; Senderowicz, 2001a, b; Ortega *et al*., 2002; Schoepfer *et al*., 2002; Senderowicz, 2002a, b; Mettey *et al*., 2003).

Cdk modulators in clinical trials

Flavopiridol

Mechanism of antiproliferative effects Flavopiridol (L86-8275 or HMR 1275, see Figure 3) is a semisynthetic flavonoid derived from rohitukine, an indigenous plant from India. Initial studies with this flavonoid revealed clear evidence of G_1/S or G_2/M arrest, due to loss in cdk1 and cdk2 (Kaur *et al*., 1992; Worland *et al*., 1993; Losiewicz *et al*., 1994). Studies using purified cdks showed that the inhibition observed is reversible and competitively blocked by ATP, with a K_i of 41 nM (Kaur

Table 1 Direct cdk modulators

Specificity against cdk's	*Examples and references*
Cdk1/cdk2/cdk5	Roscovitine and CYC202 (Meijer *et al*., 1997; Gray *et al*., 1999; McClue *et al*., 2002) Olomucine (Buquet-Fagot *et al*., 1997; Schutte *et al*., 1997; Gray *et al*., 1999) CVT-313 (Brooks *et al*., 1997) Butyrolactone I (Kitagawa *et al*., 1993) Purvalanol (Gray *et al*., 1998; Rosania *et al*., 1999) BMS-387032 (Kim *et al*., 2002) Aloisines (Mettey *et al*., 2003) Indirubins (Leclerc *et al*., 2001) Hymenialdisine (Meijer, 2000) Pyrazolo-piridines (Misra *et al*., 2003) Pyrazolo-quinoxalines (Ortega *et al*., 2002) Indenopyrazoles (Nugiel *et al*., 2001) SU9516 (Lane *et al*., 2001) Nitrosopirimidines (Mesguiche *et al*., 2003)
Cdk4	Pyrrolo-carbazoles (Zhu *et al*., 2003a) Indolocarbazoles (Zhu *et al*., 2003b) Tryaminopyrimidine (Soni *et al*., 2001) Fascaplysin (Soni *et al*., 2000) PD0183812 (Fry *et al*., 2001) Cynnamaldehydes (Jeong *et al*., 2000) Dioxobenzothiazoles (Ryu *et al*., 2000)
Nonspecific cdk	Flavopiridol (Senderowicz, 1999; Senderowicz and Sausville, 2000; Senderowicz, 2002b) Staurosporine (Akinaga *et al*., 1991; Senderowicz, 1999) UCN-01 (Akinaga *et al*., 1991, 1994; Seynaeve *et al*., 1993, 1993; Senderowicz and Sausville, 2000) Oxyndoles (Kent *et al*., 1999; Bramson *et al*., 2001; Dermatakis *et al*., 2003) Quinazolines (Sielecki *et al*., 2001)
Unknown	Toyocamycin (Park *et al*., 1996) Paullones (Gray *et al*., 1999; Schultz *et al*., 1999; Zaharevitz *et al*., 1999; Lahusen *et al*., 2003) Myricetin (Walker, 1998)

Table 2 Indirect cdk modulators

Mechanism for loss in cdk activity	*Examples and references*
ATP-binding pocket competition	Direct cdk inhibitors (see Table 1)
Endogenous CKI	
Overexpression by	$P16^{ink4a}$ (Jin *et al.*, 1995; Chintala *et al.*, 1997)
Gene therapy	$P53/p21^{cip1}$ (Eastham *et al.*, 1995; Eicher *et al.*, 1996; Spitz *et al.*, 1996; Sandig *et al.*, 1997)
	$P27^{kip1}$ (Craig *et al.*, 1997; Katayose *et al.*, 1997)
Small molecules	Lovastatin (Gray-Bablin *et al.*, 1997)
	Rapamycin (Hashemolhosseini *et al.*, 1998)
	UCN-01 (Patel, 2002a, b #7367); Akiyama, 1997#1507)
Peptidomimetic based	Perifosine (Patel *et al.*, 2002b)
	P16-derived peptides (Fahraeus *et al.*, 1996; Gius *et al.*, 1999)
	P21-derived peptides (Warbrick *et al.*, 1995; Bonfanti *et al.*, 1997)
	Peptides that block cyclin/cdk contact (Colas *et al.*, 1996; Chen *et al.*, 1999b)
Depletion of cdk/cyclins antisense approaches	Cyclin D1 antisense (Cagnoli *et al.*, 1998; Kornmann *et al.*, 1998; Wang *et al.*, 1998; Driscoll *et al.*, 1999)
Small molecules	Tamoxifene (Zhou *et al.*, 1997)
	Rapamycin (Hashemolhosseini *et al.*, 1998; Muise-Helmericks *et al.*, 1998)
	Lovastatin (Choi *et al.*, 1997; Gray-Bablin *et al.*, 1997)
	Retinoids (Wilcken *et al.*, 1996)
	Flavopiridol (Carlson *et al.*, 1999)
Modulation of proteasomal machinery	PS341 (Adams *et al.*, 2000)
Modulation of upstream phosphatases/kinases	Caffeine (Fingert *et al.*, 1988)
	Fostriecin (Roberge *et al.*, 1994)
	Dysidiolide (Blanchard *et al.*, 1999)
	Others (Baratte *et al.*, 1992; Dodo *et al.*, 2000)

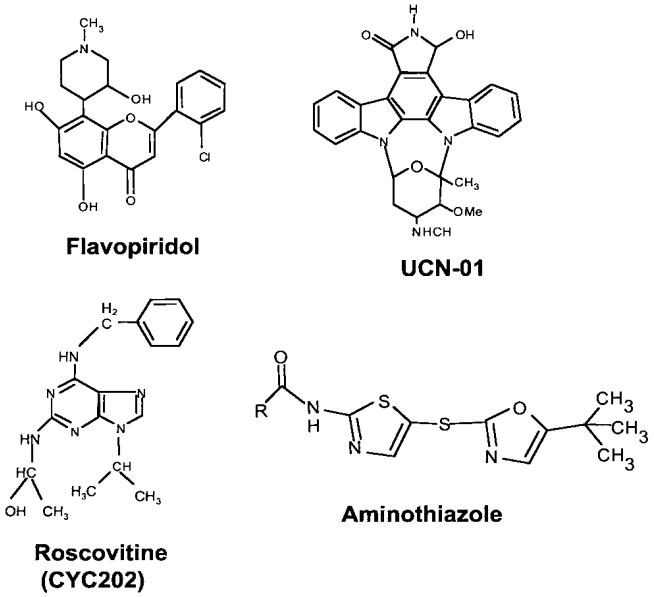

Figure 3 Structure of small-molecule direct cdk modulators in clinical trials

et al., 1992; Worland *et al.*, 1993; Losiewicz *et al.*, 1994; Carlson *et al.*, 1996a, b). Furthermore, the crystal structure of the complex of deschloroflavopiridol and cdk2 showed that flavopiridol binds to the ATP-binding pocket, with the benzopyran occupying the same region as the purine ring of ATP (De Azevedo *et al.*, 1996), confirming the earlier biochemical studies with flavopiridol (Losiewicz *et al.*, 1994). Flavopiridol inhibits all cdks thus far examined ($IC_{50} \sim 100$ nM), but it inhibits cdk7 (CAK) less potently ($IC_{50} \sim 300$ nM) (Losiewicz *et al.*, 1994; Carlson *et al.*, 1996a, b).

In addition to directly inhibiting cdks, flavopiridol promotes a decrease in the level of cyclin D1, an oncogene that is overexpressed in many human neoplasias. Of note, neoplasms that overexpress cyclin D1 have a poor prognosis (Michalides *et al.*, 1995; Fredersdorf *et al.*, 1997; Gansauge *et al.*, 1997). Depletion of cyclin D1 appears to lead to the loss of cdk activity (Carlson *et al.*, 1999). Cyclin D1 decrease is caused by depletion of cyclin D1 mRNA and was associated with a specific decline in cyclin D1 promoter, measured by a luciferase reporter assay (Carlson *et al.*, 1999). The transcriptional repression of cyclin D1 observed after treatment with flavopiridol is consistent with the effects of flavopiridol on yeast cells (see above) and underscores the conserved effect of flavopiridol on eucaryotic cyclin transcription (Gray *et al.*, 1998). In summary, flavopiridol can induce cell cycle arrest by at least three mechanisms: (1) direct inhibition of cdk activities by binding to the ATP-binding site; (2) prevention of the phosphorylation of cdks at threonine-160/161 by inhibition of cdk7/cyclin H (Worland *et al.*, 1993; Carlson *et al.*, 1996a); and (3) decrease in the amount of cyclin D1, an important cofactor for cdk4 and cdk6 activation (G_1/S arrest only).

In part, flavopiridol regulates transcription due to potent inhibition of P-TEFb (also known as cdk9/cyclin T), with a K_i of 3 nM, leading to inhibition of transcription by RNA polymerase II by blocking the transition into productive elongation. Interestingly, in contrast with all cdk's tested so far, flavopiridol was not competitive with ATP in this reaction. P-TEFb is a required cellular cofactor for the human immunodefi-

ciency virus (HIV-1) transactivator, Tat. Consistent with its ability to inhibit P-TEFb, flavopiridol blocked Tat transactivation of the viral promoter *in vitro*. Furthermore, flavopiridol blocked HIV-1 replication in both single-round and viral spread assays with an IC_{50} of less than 10 nM (Chao *et al.*, 2000). These actions of the drug led to the testing of flavopiridol through clinical trials for patients with HIV-related malignancies (Wright *et al.*, 1998).

An important biochemical effect involved in the antiproliferative activity of flavopiridol is the induction of apoptotic cell death. Hematopoietic cell lines are often quite sensitive to flavopiridol-induced apoptotic cell death, (Konig *et al.*, 1997; Arguello *et al.*, 1998; Byrd *et al.*, 1998; Parker *et al.*, 1998), but the mechanism(s) by which flavopiridol induces apoptosis have not yet been elucidated. Flavopiridol does not modulate topoisomerase I/II activity (Parker *et al.*, 1998). In certain hematopoietic cell lines, neither BCL-2/BAX nor p53 appeared to be affected (Parker *et al.*, 1998; Shapiro *et al.*, 1999b), whereas, in other systems, BCL-2 may be inhibited (Konig *et al.*, 1997). Preliminary evidence from one laboratory demonstrated that flavopiridol-induced apoptosis in leukemia cells is associated with early activation of the MAPK protein kinase family of proteins (MEK, p38 and JNK) (Lahusen *et al.*, 2000). This activation may lead to the activation of caspases (Lahusen *et al.*, 2000). As seen in this and other models, caspase inhibitors prevent flavopiridol-induced apoptosis (Byrd *et al.*, 1998; Lahusen *et al.*, 2000). It is unclear whether the putative flavopiridol-induced inhibition of cdk activity is required for induction of apoptosis.

Clear evidence of cell cycle arrest along with apoptosis was observed in a panel of squamous head and neck cancer cell lines, including a cell line (HN30) that is refractory to several DNA-damaging agents, such as γ-irradiation and bleomycin (Patel *et al.*, 1998). Again, the apoptotic effect was independent of p53 status and was associated with the depletion of cyclin D1 (Patel *et al.*, 1998). These findings have been corroborated in other preclinical models (Bible and Kaufmann, 1996; Schrump *et al.*, 1998; Chien *et al.*, 1999; Shapiro *et al.*, 1999b). Efforts to understand flavopiridol-induced apoptosis are under intense investigation.

Flavopiridol targets not only tumor cells but also angiogenesis pathways. Brusselbach *et al.* (1998) incubated primary human umbilical vein endothelial cells (HUVECs) with flavopiridol and observed apoptotic cell death even in cells that were not cycling, leading to the notion that flavopiridol may have antiangiogenic properties due to endothelial cytotoxicity. In other model systems, Kerr *et al.* (1999) tested flavopiridol in an *in vivo* Matrigel model of angiogenesis and found that flavopiridol decreased blood vessel formation, a surrogate marker for the antiangiogenic effect of this compound. Furthermore, as mentioned earlier, Melillo *et al.* (1999) demonstrated that, at low nanomolar concentrations, flavopiridol prevented the induction of vascular endothelial growth factor (VEGF) by hypoxic conditions in human monocytes. This effect was caused by a decreased stability of VEGF mRNA, which paralleled the decline in VEGF protein. Thus, the antitumor activity of flavopiridol observed may be in part due to antiangiogenic effects. Whether the various antiangiogenic actions of flavopiridol result from its interaction with a cdk target or other targets requires further study.

The antitumor effect observed with flavopiridol can also be explained by activation of differentiation pathways. It became clear recently that cells become differentiated when exit of the cell cycle (G_0) and loss of cdk2 activity occur. Based on this information, Lee *et al.* (1999) tested flavopiridol and roscovitine, both known cdk2 inhibitors, to determine if they induce a differentiated phenotype. For this purpose, NCI-H358 lung carcinoma cell lines were exposed to cdk2 antisense construct, flavopiridol, or roscovitine. Clear evidence of mucinous differentiation along with loss in cdk2 activity was observed in this lung carcinoma model. Thus, it is plausible that the antitumor effect of flavopiridol in lung carcinoma models may be due to induction of differentiation, among others (Lee *et al.*, 1999).

Several investigators have attempted to determine if flavopiridol has synergistic effects with standard chemotherapeutic agents. For example, synergistic effects in A549 lung carcinoma cells were demonstrated when treatment with flavopiridol followed treatment with paclitaxel, cytarabine, topotecan, doxorubicin, or etoposide (Bible and Kaufmann, 1997; Schwartz *et al.*, 1997). In contrast, a synergistic effect was observed with 5-fluorouracil only when cells were treated with flavopiridol for 24 h before the addition of 5-fluorouracil. Furthermore, synergistic effects with cisplatin were not schedule dependent (Bible and Kaufmann, 1997). However, Chien *et al.* (1999) failed to demonstrate a synergistic effect between flavopiridol and cisplatin and/or γ-irradiation in bladder carcinoma models. One important issue to mention is that most of these studies were performed in *in vitro* models. Thus, confirmatory studies in *in vivo* animal models are needed.

Experiments using colorectal (Colo205) and prostate (LnCaP/DU-145) carcinoma xenograft models in which flavopiridol was administered frequently over a protracted period demonstrated that flavopiridol is cytostatic (Sedlacek *et al.*, 1996; Drees *et al.*, 1997). These demonstrations led to human clinical trials of flavopiridol administered as a 72-h continuous infusion every 2 weeks (Senderowicz *et al.*, 1998b) (see below). Subsequent studies in human leukemia/lymphoma xenografts demonstrated that flavopiridol administered intravenously as a bolus rendered animals tumor- free, whereas flavopiridol administered as an infusion only delayed tumor growth (Arguello *et al.*, 1998). Moreover, in HN-12 head and neck cancer xenografts flavopiridol administered intraperitoneally for 5 days demonstrated a substantial growth delay (Patel *et al.*, 1998). Again, apoptotic cell death and cyclin D1 depletion were observed in tissues from xenografts treated with flavopiridol (Arguello *et al.*, 1998). Based on these results, a phase I trial of 1 h daily infusional flavopiridol every 3 weeks has been conducted at the NCI (Tan *et al.*, 2002).

Clinical experience with flavopiridol Two phase I clinical trials of flavopiridol administered as a 72-h continuous infusion every 2 weeks have been completed (Senderowicz *et al.*, 1998b; Thomas *et al.*, 2002). In the NCI phase I trial ($N=76$) of infusional flavopiridol, dose-limiting toxicity (DLT) was secretory diarrhea with a maximal-tolerated dose (MTD) of 50 mg/m^2/day for 3 days. In the presence of antidiarrheal prophylaxis (a combination of cholestyramine and loperamide), patients tolerated higher doses, defining a second MTD, 78 mg/m^2/day for 3 days. The DLT observed at the higher dose level was a substantial proinflammatory syndrome (fever, fatigue, local tumor pain, and modulation of acute-phase reactants) and reversible hypotension (Senderowicz *et al.*, 1998b). Minor responses were observed in patients with non-Hodgkin's lymphoma, colon, and kidney cancer for more than 6 months. Moreover, one patient with refractory renal cancer achieved a partial response for more than 8 months (Senderowicz *et al.*, 1998b). Of 14 patients who received flavopiridol for more than 6 months, five patients received flavopiridol for more than 1 year and one patient received flavopiridol for more than 2 years (Senderowicz *et al.*, 1998b). Plasma concentrations of 300–500 nM flavopiridol, which inhibit cdk activity *in vitro*, were safely achieved during this trial (Senderowicz *et al.*, 1998b).

In a complementary phase I trial also exploring the same schedule (72-h continuous infusion every 2 weeks), Thomas *et al* (2002) found that the DLT was diarrhea, corroborating the NCI experience. Moreover, plasma concentrations of 300–500 nM flavopiridol were also observed. Interestingly, there was one patient in this trial with refractory metastatic gastric cancer that progressed after a treatment regimen containing 5-fluorouracil. When treated with flavopiridol, this patient achieved a sustained complete response without any evidence of disease for more than 2 years after treatment was completed.

The first phase I trial of a daily 1-h infusion of flavopiridol for 5 consecutive days every 3 weeks was recently completed (Senderowicz *et al.*, 1998b; Tan *et al.*, 2002). This schedule was based on antitumor results observed in leukemia/lymphoma and head and neck cancer xenografts treated with flavopiridol (Arguello *et al.*, 1998; Patel *et al.*, 1998). A total of 55 patients were treated in this trial. The recommended phase II dose is 37.5 mg/m^2/day for 5 consecutive days. DLTs observed at 52.5 mg/m^2/day are nausea/vomiting, neutropenia, fatigue, and diarrhea (Senderowicz *et al.*, 1998b; Tan *et al.*, 2002). Other (nondose-limiting) side effects are local tumor pain and anorexia. To reach higher flavopiridol concentrations, the protocol was amended to administer flavopiridol for 3 days and then for 1 day only. With these protocol modifications, we were able to achieve concentrations ($\sim 4\,\mu$M) necessary to induce apoptosis in xenograft models (Arguello *et al.*, 1998; Patel *et al.*, 1998; Senderowicz *et al.*, 2000; Tan *et al.*, 2002) Of note, the half-life observed in this trial is much shorter (~ 3 h) than the infusional trial (~ 10 h). Thus, the high micromolar concentrations achieved in the 1-h infusional trial could be maintained only for short periods of time (Senderowicz *et al.*, 2000; Tan *et al.*, 2002). Several phase II trials in patients with refractory head and neck cancer, chronic lymphocytic leukemia (CLL), and mantle cell lymphoma (MCL) are currently being tested using this schedule (see below). A phase I trial testing the combination of paclitaxel and infusional (24 h) flavopiridol demonstrated good tolerability with a dose-limiting pulmonary toxicity (Schwartz *et al.*, 2002).

Phase II trials of flavopiridol given as a 72-h continuous infusion with the MTD in the absence of antidiarrheal prophylaxis (50 mg/m^2/day) to patients with CLL, non-small-cell lung cancer (see below), non-Hodgkin's lymphoma, and colon, prostate, gastric, head and neck, and kidney cancer, and phase I trials of flavopiridol administered on novel schedules and in combination with standard chemotherapeutic agents are being performed (Werner *et al.*, 1998; Wright *et al.*, 1998; Bennett *et al.*, 1999; Shapiro *et al.*, 1999a; Stadler *et al.*, 2000).

In a recently published phase II trial of flavopiridol in metastatic renal cancer, two objective responses (response rate = 6%, 95% confidence interval, 1–20%) were observed. Most patients developed grade 1–2 diarrhea and asthenia (Stadler *et al.*, 2000). In this trial, patients who demonstrated glucuronide flavopiridol metabolites in plasma have less pronounced diarrhea in comparison to nonmetabolizers (Innocenti *et al.*, 2000). Thus, it may be possible that patients with higher metabolic rates may tolerate higher doses of flavopiridol. Phase II trials of shorter (1-h) infusional flavopiridol are being conducted in MCL, CLL, and HNSCC. Of interest, several refractory CLL and MCL patients demonstrated evidence of clinical responses (partial responses) in these trials (Jose Ramon Suarez, personal communications).

Based on the interesting results obtained in several preclinical lung carcinoma models, a phase II trial of patients with metastatic lung carcinoma was performed (Shapiro *et al.*, 2001). A total of 20 patients were treated with a 72-h continuous infusion of flavopiridol every 14 days at a dose of 50 mg/m^2/day. The most common toxicities included grade $\leqslant 2$ diarrhea, asthenia, and venous thromboses. The median overall survival for the 20 patients who received treatment was 7.5 months. Of note, the median survival of 7.5 months achieved with flavopiridol is similar to the range of 7.4–8.2 months reported recently in a randomized trial of four chemotherapy regimens containing platinum analogues in combination with taxanes or gemcitabine (Schiller *et al.*, 2002). Moreover, a similar overall survival was obtained with gefitinib (Iressa), a recently approved EGFR inhibitor for the treatment of advanced lung cancer (Kris *et al.*, 2002). Based on these encouraging results, a phase III trial comparing standard combination chemotherapy versus combination chemotherapy plus flavopiridol is currently under investigation.

UCN-01

Mechanism of antiproliferative activity

Staurosporine is a potent nonspecific protein and tyrosine kinase inhibitor, with a very low therapeutic index in animals (Tamaoki, 1991). Thus, efforts to find staurosporine analogues of staurosporine have identified compounds specific for protein kinases. One staurosporine analogue, UCN-01 (see Figure 3), has potent activity against several protein kinase C isoenzymes, particularly the Ca^{2+}-dependent protein kinase C with an $IC_{50} \sim 30$ nM (Seynaeve *et al.*, 1994; Takahashi *et al.*, 1987, 1989). In addition to its effects on protein kinase C, UCN-01 has antiproliferative activity in several human tumor cell lines (Akinaga *et al.*, 1991, 1994; Seynaeve *et al.*, 1993; Wang *et al.*, 1995; Akiyama *et al.*, 1997). These effects appear not to be related to the effects of UCN-01 in PKC signaling (Wang *et al.*, 1995).

Another interesting feature, again unrelated to PKC, is 'inappropriate activation' of cdk kinases in intact cells (Wang *et al.*, 1995). This phenomenon correlates with the G2 abrogation checkpoint observed with this agent. Experimental evidence suggests that DNA damage leads to cell cycle arrest to allow DNA repair. In the presence of UCN-01, irradiated cells are unable to accumulate in the G_2 phase with subsequent early mitosis with the onset of apoptotic cell death (Wang *et al.*, 1996). The accelerated mitosis is due to activation of cdc2 kinase. These activations could be partially explained by the inactivation of Wee1, the kinase that negatively regulates the G_2/M phase transition (Yu *et al.*, 1998). Moreover, UCN-01 can have a direct effect on chk1, the protein kinase that regulates the G2 checkpoint (Sarkaria *et al.*, 1999; Busby *et al.*, 2000; Graves *et al.*, 2000). Thus, although UCN-01 at high concentrations can directly inhibit cdks *in vitro*, UCN-01 can modulate cellular 'upstream' regulators at much lower concentration, leading to inappropriate cdc2 activation. Studies from other groups suggest that not only is UCN-01 able to abrogate the G_2 checkpoint induced by DNA-damaging agents but also, in some circumstances, UCN-01 is able to abrogate the DNA damage-induced S phase checkpoint (Bunch and Eastman, 1997; Shao *et al.*, 1997).

Another interesting property of UCN-01 is its ability to arrest cells in the G_1 phase of the cell cycle (Seynaeve *et al.*, 1993; Akinaga *et al.*, 1994; Kawakami *et al.*, 1996; Shimizu *et al.*, 1996; Akiyama *et al.*, 1997; Akiyama *et al.*, 1999; Chen *et al.*, 1999a; Usuda *et al.*, 2000). When human epidermoid carcinoma A431 cells (mutated p53) or HN12 head and neck carcinoma cell lines are incubated with UCN-01, these cells were arrested in the G_1 phase with Rb hypophosphorylation and $p21^{waf1}$/$p27^{kip1}$ accumulation (Akiyama *et al.*, 1997; Patel *et al.*, 2002a). Chen *et al.* (1999a) suggest that Rb, but not p53, function is essential for UCN-01-mediated G1 arrest. However, Shimizu *et al.* (1996) demonstrated that lung carcinoma cell lines with either absent, mutant, or wild-type Rb exposed to UCN-01 displayed G_1 arrest and antiproliferative effects irrespective of Rb function. Thus, the exact role of Rb or p53 in the G_1arrest induced by UCN-01 is still unknown. Further studies on the putative target(s) for UCN-01 in the G_1 phase arrest of cells are warranted. UCN-01 demonstrated enhanced cytotoxicity in cells with mutant p53 (Wang *et al.*, 1996).

In CA-46 and HT-29 tumor cell lines carrying mutated p53 genes, potent cytotoxicity results following exposure to UCN-01. To extend these observations further, the MCF-7 cell line with no endogenous p53 because of the ectopic expression of E6, a human papillomavirus type-16 protein, showed enhanced cytotoxicity when treated with a DNA-damaging agent, such as cisplatin, and UCN-01, compared with the isogenic wild-type MCF-7 cell line. Thus, a common feature observed in more than 50% of human neoplasias, associated with poor outcome and refractoriness to standard chemotherapies (Marchetti *et al.*, 1993; Lowe *et al.*, 1994), may render tumor cells more sensitive to UCN-01.

A very exciting recent development is the discovery that UCN-01 can modulate the PI3 kinase/AKT survival pathway (Testa and Bellacosa, 2001; Sato *et al.*, 2002). UCN-01 displays a potent inhibition *in vitro* of the pdk1 serine/threonine kinase, leading to dephosphorylation and inactivation of akt (Sato *et al.*, 2002). Although this is an exciting novel feature of UCN-01, it is of utmost importance to demonstrate whether the antitumor effects of UCN-01 are mediated by this action. Moreover, demonstration that these effects also occur in *in vivo* settings is crucial.

As previously mentioned, synergistic effects of UCN-01 have been observed with many chemotherapeutic agents, including mitomycin C, 5-fluorouracil, carmustine, and camptothecin, among others (Akinaga *et al.*, 1993; Bunch and Eastman, 1996; Pollack *et al.*, 1996; Husain *et al.*, 1997; Shao *et al.*, 1997; Tsuchida and Urano, 1997; Hsueh *et al.*, 1998; Jones *et al.*, 2000; Sugiyama *et al.*, 2000). Therefore, it is possible that combining UCN-01 with these or other agents could improve its therapeutic index. Clinical trials exploring these possibilities are currently being developed.

UCN-01 administered by an intravenous or intraperitoneal route displayed antitumor activity in xenograft model systems with breast carcinoma (MCF-7 cells), renal carcinoma (A498 cells), and leukemia (MOLT-4 and HL-60) cells (A Senderowicz, unpublished results). The antitumor effect was greater when UCN-01 was given over a longer period. This requirement for a longer period of treatment was also observed in *in vitro* models, with the greatest antitumor activity observed when UCN-01 was present for 72 h (Seynaeve *et al.*, 1993). Thus, a clinical trial using a 72-h continuous infusion every 2 weeks was conducted.

Clinical trials of UCN-01

The first phase I trial of UCN-01 was recently completed (Senderowicz *et al.*, 1998a; Sausville *et al.*, 2001). UCN-01 was initially administered as a 72-h continuous infusion every 2 weeks based on data from *in vitro* and

xenograft preclinical models. However, it became apparent in the first few patients that the drug had an unexpectedly long half-life (~30 days). This half-life was 100 times longer than the half-life observed in preclinical models, most likely due to the avid binding of UCN-01 to α_1-acid glycoprotein (Fuse *et al.*, 1998; Sausville *et al.*, 1998). Thus, the protocol was modified to administer UCN-01 every 4 weeks (one half-life) and in subsequent courses, the duration of infusion was decreased by half (total 36 h). Thus, it was possible to reach similar peak plasma concentrations in subsequent courses with no evidence of drug accumulation. There was no evidence of myelotoxicity or gastrointestinal toxicity (prominent side effects observed in animal models), despite very high plasma concentrations achieved (35–50 μM) (Fuse *et al.*, 1998; Sausville *et al.*, 1998, 2001; Senderowicz *et al.*, 1998a). DLTs were nausea/vomiting (amenable to standard antiemetic treatments), symptomatic hyperglycemia associated with an insulin-resistance state (increase in insulin and c-peptide levels while receiving UCN-01), and pulmonary toxicity characterized by substantial hypoxemia without obvious radiologic changes. The recommended phase II dose of UCN-01 given on a 72-h continuous infusion schedule was 42.5 mg/m^2/day (Sausville *et al.*, 2001). One patient with refractory metastatic melanoma developed a partial response that lasted 8 months. Another patient with refractory anaplastic large-cell lymphoma that had failed multiple chemotherapeutic regimens including high-dose chemotherapy has no evidence of disease more than 4 years after the initiation of UCN-01. Moreover, a few patients with leiomyosarcoma, non-Hodgkin's lymphoma, and lung cancer demonstrated stable disease for $\geqslant$6 months (Senderowicz *et al.*, 1999; Sausville *et al.*, 2001).

In order to estimate 'free UCN-01 concentrations' in body fluids, several efforts were considered. Plasma ultracentrifugation and salivary determination of UCN-01 revealed similar results. At the recommended phase II dose (37.5 mg/m^2/day over 72 h), concentrations of 'free-salivary' UCN-01 (~100 nM) that may cause G_2 checkpoint abrogation can be achieved. As mentioned earlier, UCN-01 is a potent PKC inhibitor. In order to determine the putative signaling effects of UCN-01 in tissues, bone marrow aspirates and tumor cells were obtained from patients before and during the first cycle of UCN-01 administration. Western blot studies were performed in those samples against phosphorylated adducin, a cytoskeletal membrane protein, a specific substrate phosphorylated by PKC (Fowler *et al.*, 1998). A clear loss in phosphoadducin content in the post-treatment samples was observed in all tumor and bone marrow samples tested, concluding that UCN-01 can modulate PKC activity in tissues from patients in this trial (Senderowicz *et al.*, 1999; Sausville *et al.*, 2001).

Several groups are conducting shorter duration (3-h) infusional trials of UCN-01. Interestingly, the toxicity profile of shorter infusions is similar to the toxicities observed with the 72-h infusion trial (Tamura *et al.*, 1999; Dees *et al.*, 2000). However, with shorter infusions, more pronounced hypotension was observed (Tamura *et al.*, 1999; Dees *et al.*, 2000). The determination of free UCN-01 in these trials is of utmost importance as higher free concentrations for shorter periods may be more or less beneficial compared with the free concentrations observed in the 72-h infusion trial.

Based on the unique pharmacological features and anecdotal clinical evidence of synergistic effects in one patient with refractory disease (Wilson *et al.*, 2000), several combination trials with standard chemotherapeutic agents recently commenced. A phase I/II trial of gemcitabine followed by 72-h infusional UCN-01 in CLL started at the NCI. Other studies of UCN-01 in combination with cisplatin, 5-fluorouracil, among other agents, also commenced recently.

Summary

Most human malignancies have an aberration in the Rb pathway due to 'cdk hyperactivation'. Several small-molecule cdk modulators are being discovered and tested in the clinic. The first ATP competitive cdk inhibitors tested in clinical trials, flavopiridol and UCN-01, showed promising results with evidence of antitumor activity and plasma concentrations sufficient to inhibit cdk-related functions. The best schedule to be administered, combination with standard chemotherapeutic agents, best tumor types to be targeted, and demonstration of cdk modulation from tumor samples from patients in these trials are important issues that need to be answered in order to advance these agents to the clinical arena.

References

Aagaard L, Lukas J, Bartkova J, Kjerulff AA, Strauss M and Bartek J. (1995). *Int. J. Cancer,* **61,** 115–120.

Adams J, Palombella VJ and Elliott PJ. (2000). *Invest. New Drugs,* **18,** 109–121.

Akinaga S, Gomi K, Morimoto M, Tamaoki T and Okabe M. (1991). *Cancer Res,* **51,** 4888–4892.

Akinaga S, Nomura K, Gomi K and Okabe M. (1993). *Cancer Chemother. Pharmacol,* **32,** 183–189.

Akinaga S, Nomura K, Gomi K and Okabe M. (1994). *Cancer Chemother. Pharmacol.,* **33,** 273–280.

Akiyama T, Shimizu M, Okabe M, Tamaoki T and Akinaga S. (1999). *Anticancer Drugs,* **10,** 67–78.

Akiyama T, Yoshida T, Tsujita T, Shimizu M, Mizukami T, Okabe M and Akinaga S. (1997). *Cancer Res.,* **57,** 1495–1501.

An HX, Beckmann MW, Reifenberger G, Bender HG and Niederacher D. (1999). *Am. J. Pathol.,* **154,** 113–118.

Arguello F, Alexander M, Sterry J, Tudor G, Smith E, Kalavar N, Greene J, Koss W, Morgan D, Stinson S, Siford T, Alvord W, Labansky R and Sausville E. (1998). *Blood,* **91,** 2482–2490.

Baratte B, Meijer L, Galaktionov K and Beach D. (1992). *Anticancer Res.*, **12,** 873–880.
Bartek J, Staskova Z, Draetta G and Lukas J. (1993). *Stem Cells (Dayt)*, **11** (Suppl. 1), 51–58.
Bartkova J, Lukas J, Guldberg P, Alsner J, Kirkin AF, Zeuthen J and Bartek J. (1996). *Cancer Res.*, **56,** 5475–5483.
Bartkova J, Lukas J, Muller H, Strauss M, Gusterson B and Bartek J. (1995). *Cancer Res.*, **55,** 949–956.
Bennett S, Mani S, O'Reilly S, Wright J, Schilsky R, Vokes E and Grochow L. (1999). *Proc. Am. Soc. Clin. Oncol.*, **18**, 156.
Bible KC and Kaufmann SH. (1996). *Cancer Res.*, **56,** 4856–4861.
Bible KC and Kaufmann SH. (1997). *Cancer Res.*, **57,** 3375–3380.
Blanchard JL, Epstein DM, Boisclair MD, Rudolph J and Pal K. (1999). *Bioorg. Med. Chem. Lett.*, **9,** 2537–2538.
Bonfanti M, Taverna S, Salmona M, D'Incalci M and Broggini M. (1997). *Cancer Res.*, **57,** 1442–1446.
Bramson HN, Corona J, Davis ST, Dickerson SH, Edelstein M, Frye SV, Gampe Jr RT, Harris PA, Hassell A, Holmes WD, Hunter RN, Lackey KE, Lovejoy B, Luzzio MJ, Montana V, Rocque WJ, Rusnak D, Shewchuk L, Veal JM, Walker DH and Kuyper LF. (2001). *J. Med. Chem.*, **44,** 4339–4358.
Brooks EE, Gray NS, Joly A, Kerwar SS, Lum R, Mackman RL, Norman TC, Rosete J, Rowe M, Schow SR, Schultz PG, Wang X, Wick MM and Shiffman D. (1997). *J. Biol. Chem.*, **272,** 29207–29211.
Brusselbach S, Nettelbeck DM, Sedlacek HH and Muller R. (1998). *Int. J. Cancer*, **77,** 146–152.
Bunch RT and Eastman A. (1996). *Clin. Cancer Res.*, **2,** 791–797.
Bunch RT and Eastman A. (1997). *Cell Growth Differ.*, **8,** 779–788.
Buquet-Fagot C, Lallemand F, Montagne M and Mester J. (1997). *Anti-Cancer Drugs*, **8,** 623–631.
Busby EC, Leistritz DF, Abraham RT, Karnitz LM and Sarkaria JN. (2000). *Cancer Res.*, **60,** 2108–2112.
Byrd JC, Shinn, C, Waselenko JK, Fuchs EJ, Lehman TA, Nguyen PL, Flinn IW, Diehl LF, Sausville E and Grever MR. (1998). *Blood*, **92,** 3804–3816.
Cagnoli M, Barbieri F, Bruzzo C and Alama A. (1998). *Gynecol. Oncol.*, **70,** 372–377.
Carlson BA, Dubay MM, Sausville EA, Brizuela L and Worland PJ. (1996b). *Cancer Res.*, **56,** 2973–2978.
Carlson B, Lahusen T, Singh S, Loaiza-Perez A, Worland PJ, Pestell R, Albanese C, Sausville EA and Senderowicz AM. (1999). *Cancer Res.*, **59,** 4634–4641.
Carlson B, Pearlstein R, Naik R, Sedlacek H, Sausville E and Worland P. (1996a). *Proc. Am. Assoc. Cancer Res.*, **37,** 424.
Chao SH, Fujinaga K, Marion JE, Taube R, Sausville EA, Senderowicz AM, Peterlin BM and Price DH. (2000). *J. Biol. Chem.*, **275,** 28345–28348.
Chen X, Lowe M and Keyomarsi K. (1999a). *Oncogene*, **18,** 5691–5702.
Chen YN, Sharma SK, Ramsey TM, Jiang L, Martin MS, Baker K, Adams PD, Bair KW and Kaelin Jr WG. (1999b). *Proc. Natl. Acad. Sci. USA*, **96,** 4325–4329.
Chien M, Astumian M, Liebowitz D, Rinker-Schaeffer C and Stadler W. (1999). *Cancer Chemother Pharmacol.*, **44,** 81–87.
Chintala SK, Fueyo J, Gomez-Manzano C, Venkaiah B, Bjerkvig R, Yung WK, Sawaya R, Kyritsis AP and Rao JS. (1997). *Oncogene*, **15,** 2049–2057.
Choi YH, Lee, SJ, Nguyen P, Jang JS, Lee J, Wu ML, Takano E, Maki M, Henkart PA and Trepel JB. (1997). *J. Biol. Chem.*, **272,** 28479–28484.
Colas P, Cohen B, Jessen T, Grishina I, McCoy J and Brent R. (1996). *Nature*, **380,** 548–550.
Craig C, Wersto R, Kim M, Ohri E, Li Z, Katayose D, Lee SJ, Trepel J, Cowan K and Seth P. (1997). *Oncogene*, **14,** 2283–2289.
De Azevedo WF, Leclerc S, Meijer L, Havlicek L, Strnad M and Kim SH. (1997). *Eur. J. Biochem.*, **243,** 518–526.
De Azevedo Jr WF, Mueller-Dieckmann HJ, Schulze-Gahmen U, Worland PJ, Sausville E and Kim SH. (1996). *Proc. Natl. Acad. Sci. USA*, **93,** 2735–2740.
Dees E, O'Reilly S, Figg W, Elza-Brown K, Aylesworth C, Carducci M, Byrd J, Grever M and Donehower R. (2000). *Proc. Am. Soc. Clin. Oncol.*, **19,** 154.
DeGregori J, Leone G, Ohtani K, Miron A and Nevins JR. (1995). *Genes Dev.*, **9,** 2873–2887.
Dermatakis A, Luk KC and DePinto W. (2003). *Bioorg. Med. Chem.*, **11,** 1873–1881.
Dodo K, Takahashi M, Yamada Y, Sugimoto Y, Hashimoto Y and Shirai R. (2000). *Bioorg. Med. Chem. Lett.*, **10,** 615–617.
Drees M, Dengler W, Roth T, Labonte H, Mayo J, Malspeis L, Grever M, Sausville E and Fiebig H. (1997). *Clin. Cancer Res.*, **32,** 273–279.
Driscoll B, Buckley S, Barsky L, Weinberg K, Anderson KD and Warburton D. (1999). *Am. J. Physiol.*, **276,** L679–L687.
Eastham JA, Hall SJ, Sehgal I, Wang J, Timme TL, Yang G, Connell-Crowley L, Elledge SJ, Zhang WW and Harper JW *et al* (1995). *Cancer Res.*, **55,** 5151–5155.
Eicher SA, Clayman GL, Liu TJ, Shillitoe EJ, Storthz KA, Roth JA and Lotan R. (1996). *Clin. Cancer Res.*, **2,** 1659–1664.
Fahraeus R, Paramio JM, Ball KL, Lain S and Lane DP. (1996). *Curr. Biol.*, **6,** 84–91.
Fingert HJ, Pu AT, Chen ZY, Googe PB, Alley MC and Pardee AB. (1988). *Cancer Res.*, **48,** 4375–4381.
Fowler L, Dong L, Bowes III RC, van de Water B, Stevens JL and Jaken S. (1998). *Cell Growth Differ.*, **9,** 177–184.
Fredersdorf S, Burns J, Milne AM, Packham G, Fallis L, Gillett CE, Royds JA, Peston D, Hall PA, Hanby AM, Barnes DM, Shousha S, O Hare MJ and Lu X. (1997). *Proc. Natl. Acad. Sci. USA*, **94,** 6380–6385.
Fry DW, Bedford DC, Harvey PH, Fritsch A, Keller PR, Wu Z, Dobrusin E, Leopold WR, Fattaey A and Garrett MD. (2001). *J. Biol. Chem.*, **276,** 16617–16623.
Fuse E, Tanii H, Kurata N, Kobayashi H, Shimada Y, Tamura T, Sasaki Y, Tanigawara Y, Lush RD, Headlee D, Figg W, Arbuck SG, Senderowicz AM, Sausville EA, Akinaga S, Kuwabara T and Kobayashi S. (1998). *Cancer Res.*, **58,** 3248–3253.
Gansauge S, Gansauge F, Ramadani M, Stobbe H, Rau B, Harada N and Beger HG. (1997). *Cancer Res.*, **57,** 1634–1637.
Gius DR, Ezhevsky SA, Becker-Hapak M, Nagahara H, Wei MC and Dowdy SF. (1999). *Cancer Res.*, **59,** 2577–2580.
Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, O'Connor PM and Piwnica-Worms H. (2000). *J. Biol. Chem.*, **275,** 5600–5605.
Gray-Bablin J, Rao S and Keyomarsi K. (1997). *Cancer Res.*, **57,** 604–609.
Gray N, Detivaud L, Doerig C and Meijer L. (1999). *Curr. Med. Chem.*, **6,** 859–875.
Gray NS, Wodicka L, Thunnissen AM, Norman TC, Kwon S, Espinoza FH, Morgan DO, Barnes G, LeClerc S, Meijer L, Kim SH, Lockhart DJ and Schultz PG. (1998). *Science*, **281,** 533–538.

Hashemolhosseini S, Nagamine Y, Morley SJ, Desrivieres S, Mercep L and Ferrari S. (1998). *J. Biol. Chem.*, **273,** 14424–14429.

Hatakeyama M and Weinberg RA. (1995). *Prog. Cell Cycle Res.*, **1,** 9–19.

Hsueh CT, Kelsen D and Schwartz GK. (1998). *Clin. Cancer Res.*, **4,** 2201–2206.

Hunter T and Pines J. (1994). *Cell,* **79,** 573–582.

Husain A, Yan XJ, Rosales N, Aghajanian C, Schwartz GK and Spriggs DR. (1997). *Clin. Cancer Res.*, **3,** 2089–2097.

Ichimura K, Schmidt EE, Goike HM and Collins VP. (1996). *Oncogene,* **13,** 1065–1072.

Innocenti F, Stadler WM, Iyer L, Ramirez J, Vokes E and Ratain M. (2000). *Clin. Cancer Res.*, **6,** 3400–3405.

Jacks T and Weinberg RA. (1998). *Science,* **280,** 1035–1036.

Jeong HW, Kim MR, Son KH, Han MY, Ha JH, Garnier, M, Meijer L and Kwon BM. (2000). *Bioorg. Med. Chem. Lett.*, **10,** 1819–1822.

Jin X, Nguyen D, Zhang WW, Kyritsis AP and Roth JA. (1995). *Cancer Res.*, **55,** 3250–3253.

Jones CB, Clements MK, Wasi S and Daoud SS. (2000). *Cancer Chemother. Pharmacol.*, **45,** 252–258.

Kanoe H, Nakayama T, Murakami H, Hosaka T, Yamamoto H, Nakashima Y, Tsuboyama T, Nakamura T, Sasaki MS and Toguchida J. (1998). *Anticancer Res.*, **18,** 2317–2321.

Katayose Y, Kim M, Rakkar AN, Li Z, Cowan KH and Seth P. (1997). *Cancer Res.*, **57,** 5441–5445.

Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, Czech J, Naik R and Sausville E. (1992). *J. Natl. Cancer Inst.*, **84,** 1736–1740.

Kawakami K, Futami H, Takahara J and Yamaguchi K. (1996). *Biochem. Biophys. Res. Commun.*, **219,** 778–783.

Kent LL, Hull-Campbell NE, Lau T, Wu JC, Thompson SA and Nori M. (1999). *Biochem. Biophys. Res. Commun.*, **260,** 768–774.

Kerr JS, Wexler RS, Mousa SA, Robinson CS, Wexler EJ, Mohamed S, Voss ME, Devenny JJ, Czerniak PM, Gudzelak Jr A and Slee AM. (1999). *Anticancer Res.*, **19,** 959–968.

Kim KS, Kimball SD, Misra RN, Rawlins DB, Hunt JT, Xiao HY, Lu S, Qian L, Han WC, Shan W, Mitt T, Cai ZW, Poss MA, Zhu H, Sack JS, Tokarski JS, Chang CY, Pavletich N, Kamath A, Humphreys, WG, Marathe P, Bursuker I, Kellar KA, Roongta U, Batorsky R, Mulheron JG, Bol D, Fairchild CR, Lee FY and Webster KR. (2002). *J. Med. Chem.*, **45,** 3905–3927.

Kitagawa M, Okabe T, Ogino H, Matsumoto H, Suzuki-Takahashi I, Kokubo T, Higashi H, Saitoh S, Taya Y and Yasuda H *et al* (1993). *Oncogene,* **8,** 2425–2432.

Konig A, Schwartz GK, Mohammad RM, Al-Katib A and Gabrilove JL. (1997). *Blood,* **90,** 4307–4312.

Kornmann M, Arber N and Korc M. (1998). *J. Clin. Invest.*, **101,** 344–352.

Kris M, Natale N, Herbst RS, Lynch Jr TJ, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis C, Albain KS, Brahmer JR, Sandler A, Crawford J, Lutzker SG, Lilenbaum R, Helms L, Wolf M, Averbuch S, Ochs J and Kay A. (2002). *Proc. Am. Soc. Clin. Oncol.*, **21,** 1166.

Lahusen T, De Siervi A, Kunick C and Senderowicz AM. (2003). *Mol Carcinogen.*, **36,** 183–194.

Lahusen J, Loaiza-Perez A, Sausville EA and Senderowicz AM. (2000). *Proceedings of the Twentieth Annual Meeting of the American Association of Cancer Research.* San Francisco, CA.

Lane ME, Yu B, Rice A, Lipson KE, Liang C, Sun L, Tang C, McMahon G, Pestell RG and Wadler S. (2001). *Cancer Res.*, **61,** 6170–6177.

Leclerc S, Garnier M, Hoessel R, Marko D, Bibb JA, Snyder GL, Greengard P, Biernat J, Wu YZ, Mandelkow EM, Eisenbrand G and Meijer L. (2001). *J. Biol. Chem.*, **276,** 251–260.

Lee HR, Chang TH, Tebalt III MJ, Senderowicz AM and Szabo E. (1999). *Int. J. Oncol.*, **15,** 161–166.

Lees JA, Saito M, Vidal M, Valentine M, Look T, Harlow E, Dyson N and Helin K. (1993). *Mol. Cell. Biol.*, **13,** 7813–7825.

Losiewicz MD, Carlson BA, Kaur G, Sausville EA and Worland PJ. (1994). *Biochem. Biophys. Res. Commun.*, **201,** 589–5895.

Lowe SW, Bodis S, Bardeesy N, McClatchey A, Remington L, Ruley HE, Fisher DE, Jacks T, Pelletier J and Housman DE. (1994). *Cold Spring Harbor Symp. Quant. Biol.*, **59,** 419–426.

Lukas J, Aagaard L, Strauss M and Bartek J. (1995a). *Cancer Res.*, **55,** 4818–4823.

Lukas J, Bartkova J, Rohde M, Strauss M and Bartek J. (1995b). *Mol. Cell. Biol.*, **15,** 2600–2611.

Marchetti A, Buttitta F, Merlo G, Diella F, Pellegrini S, Pepe S, Macchiarini P, Chella A, Angeletti CA and Callahan R *et al* (1993). *Cancer Res.*, **53,** 2846–2851.

Masciullo V, Scambia G, Marone M, Giannitelli C, Ferrandina G, Bellacosa A, Benedetti Panici P and Mancuso S. (1997). *Int. J. Cancer,* **74,** 390–395.

McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, Fischer PM, MacKenzie M, Melville J, Stewart K, Wang S, Zhelev N, Zheleva D and Lane DP. (2002). *Int. J. Cancer,* **102,** 463–468.

Meijer L. (2000). *Drug Resist. Updat.*, **3,** 83–88.

Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, Inagaki M, Delcros JG and Moulinoux JP. (1997). *Eur. J. Biochem.*, **243,** 527–536.

Meijer L and Kim SH. (1997). *Methods Enzymol.*, **283,** 113–128.

Melillo G, Sausville EA, Cloud K, Lahusen T, Varesio L and Senderowicz AM. (1999). *Cancer Res.*, **59,** 5433–5437.

Mesguiche V, Parsons RJ, Arris CE, Bentley J, Boyle FT, Curtin NJ, Davies TG, Endicott JA, Gibson AE, Golding BT, Griffin RJ, Jewsbury P, Johnson LN, Newell DR, Noble ME, Wang LZ and Hardcastle IR. (2003). *Bioorg. Med. Chem. Lett.*, **13,** 217–222.

Mettey Y, Gompel M, Thomas V, Garnier M, Leost M, Ceballos-Picot I, Noble M, Endicott J, Vierfond JM and Meijer L. (2003). *J. Med. Chem.*, **46,** 222–236.

Michalides R, van Veelen N, Hart A, Loftus B, Wientjens E and Balm A. (1995). *Cancer Res.*, **55,** 975–978.

Misra RN, Rawlins DB, Xiao H, Shan W, Bursuker I, Kellar KA, Mulheron JG, Sack JS, Tokarski JS, Kimball SD and Webster KR. (2003). *Bioorg. Med. Chem. Lett.*, **13,** 1133–1136.

Morgan DO. (1997). *Annu. Rev. Cell. Dev. Biol.*, **13,** 261–291.

Motokura T and Arnold A. (1993). *Curr. Opin. Genet. Dev.*, **3,** 5–10.

Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN and Rosen N. (1998). *J. Biol. Chem.*, **273,** 29864–29872.

Nugiel DA, Etzkorn AM, Vidwans A, Benfield PA, Boisclair M, Burton CR, Cox S, Czerniak PM, Doleniak D and Seitz SP. (2001). *J. Med. Chem.*, **44,** 1334–1336.

Ortega MA, Montoya ME, Zarranz B, Jaso A, Aldana I, Leclerc S, Meijer L and Monge A. (2002). *Bioorg. Med. Chem.*, **10,** 2177–2184.

Park S, Cheon J, Lee Y, Park Y, Lee K, Lee C and Lee S. (1996). *Mol. Cells,* **6,** 679–683.
Parker B, Kaur G, Nieves-Neira W, Taimi M, Kolhagen G, Shimizu T, Pommier Y, Sausville E and Senderowicz AM. (1998). *Blood,* **91,** 458–465.
Patel V, Lahusen T, Leethanakul C, Igishi T, Kremer M, Quintanilla-Martinez L, Ensley JF, Sausville EA, Gutkind JS and Senderowicz AM. (2002a). *Clin. Cancer Res.,* **8,** 3549–3560.
Patel V, Lahusen T, Sy T, Sausville EA, Gutkind JS and Senderowicz AM. (2002b). *Cancer Res.,* **62,** 1401–1409.
Patel V, Senderowicz AM, Pinto D, Igishi T, Raffeld M, Quintanilla-Martinez L, Ensley JF, Sausville EA and Gutkind JS. (1998). *J. Clin. Invest.,* **102,** 1674–1681.
Pollack IF, Kawecki S and Lazo JS. (1996). *J. Neurosurg.,* **84,** 1024–1032.
Roberge M, Tudan C, Hung SM, Harder KW, Jirik FR and Anderson H. (1994). *Cancer Res.,* **54,** 6115–6121.
Robles AI, Rodriguez-Puebla ML, Glick AB, Trempus C, Hansen L, Sicinski P, Tennant RW, Weinberg RA, Yuspa SH and Conti CJ. (1998). *Genes Dev,* **12,** 2469–2474.
Rodriguez-Puebla ML, LaCava M and Conti CJ. (1999). *Cell Growth Differ.,* **10,** 467–472.
Rosania GR, Merlie Jr J, Gray N, Chang YT, Schultz PG and Heald R. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 4797–4802.
Ryu CK, Kang HY, Lee SK, Nam KA, Hong CY, Ko WG and Lee BH. (2000). *Bioorg. Med. Chem. Lett.,* **10,** 461–464.
Sandig V, Brand K, Herwig S, Lukas J, Bartek J and Strauss M. (1997). *Nat. Med.,* **3,** 313–319.
Sarkaria JN, Busby EC, Tibbetts RS, Roos P, Taya Y, Karnitz LM and Abraham RT. (1999). *Cancer Res.,* **59,** 4375–4382.
Sato S, Fujita N and Tsuruo T. (2002). *Oncogene,* **21,** 1727–1738.
Sausville EA, Arbuck SG, Messmann R, Headlee D, Bauer KS, Lush RM, Murgo A, Figg WD, Lahusen T, Jaken S, Jing X, Roberge M, Fuse E, Kuwabara T and Senderowicz AM. (2001). *J. Clin. Oncol.,* **19,** 2319–2333.
Sausville EA, Lush RD, Headlee D, Smith AC, Figg WD, Arbuck SG, Senderowicz AM, Fuse E, Tanii H, Kuwabara T and Kobayashi S. (1998). *Cancer Chemother. Pharmacol.,* **42** (Suppl), S54–S59.
Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J and Johnson DH. (2002). *N. Engl. J. Med.,* **346,** 92–98.
Schoepfer J, Fretz H, Chaudhuri B, Muller L, Seeber E, Meijer L, Lozach O, Vangrevelinghe E and Furet P. (2002). *J. Med. Chem.,* **45,** 1741–1747.
Schrump DS, Matthews W, Chen GA, Mixon A and Altorki NK. (1998). *Clin. Cancer Res.,* **4,** 2885–2890.
Schultz C, Link, A, Leost M, Zaharevitz DW, Gussio R, Sausville EA, Meijer L and Kunick C. (1999). *J. Med. Chem.,* **42,** 2909–2919.
Schutte B, Nieland L, van Engeland M, Henfling ME, Meijer L and Ramaekers FC. (1997). *Exp. Cell. Res.,* **236,** 4–15.
Schwartz G, Farsi K, Maslak P, Kelsen D and Spriggs D. (1997). *Clin. Cancer Res.,* **3,** 1467–1472.
Schwartz GK, O'Reilly E, Ilson D, Saltz L, Sharma S, Tong W, Maslak P, Stoltz M, Eden L, Perkins P, Endres S, Barazzoul J, Spriggs D and Kelsen D. (2002). *J. Clin. Oncol.,* **20,** 2157–2170.
Sedlacek HH, Czech J, Naik R, Kaur G, Worland P, Losiewicz M, Parker B, Carlson B, Smith A, Senderowicz A and Sausville E. (1996). *Int. J. Oncol.,* **9,** 1143–1168.
Senderowicz AM. (1999). *Invest. New Drugs,* **17,** 313–320.
Senderowicz AM. (2000). *Oncogene,* **19,** 6600–6606.
Senderowicz AM. (2001a). *Cancer Chemother. Biol. Response Modif.,* **19,** 165–188.
Senderowicz AM. (2001b). *Cancer Chemother. Biol. Response Modif.,* **19,** 165–188.
Senderowicz AM. (2002a). *Oncologist,* **7,** 12–19.
Senderowicz AM. (2002b). *Hematol. Oncol. Clin. N. Am.,* **16,** 1229–1253.
Senderowicz AM, Headlee D, Lush R, Bauer K, Figg W, Murgo A, S, Arbuck S, Inoue K, Kobashi S, Kuwabara T and Sausville E. (1998a). *10th National Cancer Institute-European Organisation for Research on Treatment of Cancer Symposium Proceedings*, Amsterdam, Holland, pp. 78.
Senderowicz AM, Headlee D, Lush R, Bauer K, Figg W, Murgo AS, Arbuck S, Inoue K, Kobashi S, Kuwabara T and Sausville E. (1999). *Proceedings of the Thirty-fifth Annual Meeting of the American Society of Clinical Oncology* Vol. 18, Atlanta, GA.
Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villalba L, Hill K, Steinberg SM, Figg WD, Tompkins A, Arbuck SG and Sausville EA. (1998b). *J. Clin. Oncol.,* **16,** 2986–2999.
Senderowicz AM, Messmann R, Arbuck S, Headlee D, Zhai S, Murgo A, Melillo G, Figg W and Sausville E. (2000). *Proceedings of the Annual Meeting of the American Society of Clinical Oncology, New Orleans.*
Senderowicz AM and Sausville EA. (2000). *J. Natl. Cancer Inst.,* **92,** 376–387.
Seynaeve CM, Kazanietz MG, Blumberg PM, Sausville EA and Worland PJ. (1994). *Mol. Pharmacol.,* **45,** 1207–1214.
Seynaeve CM, Stetler-Stevenson M, Sebers S, Kaur G, Sausville EA and Worland PJ. (1993). *Cancer Res.,* **53,** 2081–2086.
Shao RG, Cao CX, Shimizu T, O'Connor PM, Kohn KW and Pommier Y. (1997). *Cancer Res.,* **57,** 4029–4035.
Shapiro GI, Koestner DA, Matranga CB and Rollins BJ. (1999b). *Clin. Cancer Res.,* **5,** 2925–2938.
Shapiro G, Patterson A, Lynch C, Lucca J, Anderson L, Boral A, Elias A, Lu H, Salgia R, Skarin A, Panek-Clark C, McKenna R, Rabin M, Vasconcelles M, Eder P, Supko J, Lynch T and Rollins B. (1999a). *Proceedings of the American Society of Clinical Oncology* Vol. 18, Atlanta, GA.
Shapiro GI, Supko JG, Patterson A, Lynch C, Lucca J, Zacarola PF, Muzikansky A, Wright JJ, Lynch Jr TJ and Rollins BJ. (2001). *Clin. Cancer Res.,* **7,** 1590–1599.
Sherr CJ. (1996). *Science,* **274,** 1672–1677.
Shimizu E, Zhao MR, Nakanishi H, Yamamoto A, Yoshida S, Takada M, Ogura T and Sone S. (1996). *Oncology,* **53,** 494–504.
Sielecki TM, Johnson TL, Liu J, Muckelbauer JK, Grafstrom RH, Cox S, Boylan J, Burton CR, Chen H, Smallwood A, Chang CH, Boisclair M, Benfield PA, Trainor GL and Seitz SP. (2001). *Bioorg. Med. Chem. Lett.,* **11,** 1157–1160.
Soni R, Muller L, Furet P, Schoepfer J, Stephan C, Zumstein-Mecker S, Fretz H and Chaudhuri B. (2000). *Biochem. Biophys. Res. Commun.,* **275,** 877–884.
Soni R, O'Reilly T, Furet P, Muller L, Stephan C, Zumstein-Mecker S, Fretz H, Fabbro D and Chaudhuri B. (2001). *J. Natl. Cancer Inst.,* **93,** 436–446.
Sonoda Y, Yoshimoto T and Sekiya T. (1995). *Oncogene,* **11,** 2145–2149.
Spitz FR, Nguyen D, Skibber JM, Cusack J, Roth JA and Cristiano RJ. (1996). *Anticancer Res.,* **16,** 3415–3422.
Stadler WM, Vogelzang NJ, Amato R, Sosman J, Taber D, Liebowitz D and Vokes EE. (2000). *J. Clin. Oncol.,* **18,** 371–375.

Sugiyama K, Shimizu M, Akiyama T, Tamaoki T, Yamaguchi K, Takahashi R, Eastman A and Akinaga S. (2000). *Int. J. Cancer.*, **85,** 703–709.

Takahashi I, Kobayashi E, Asano K, Yoshida M and Nakano H. (1987). *J. Antibiot (Tokyo)*, **40,** 1782–1784.

Takahashi I, Saitoh Y, Yoshida M, Sano H, Nakano H, Morimoto M and Tamaoki T. (1989). *J. Antibiot (Tokyo)*, **42,** 571–576.

Tamaoki T. (1991). *Methods Enzymol.*, **201,** 340–347.

Tamura T, Sasaki Y, Minami H, Fujii H, Ito K, Igarashi T, Kamiya Y, Kurata T, Ohtsu T, Onozawa Y, Yamamoto N, Yamamoto N, Watanabe Y, Tanigaara Y, Fuse E, Kuwabara T, Kobayahsi S and Shimada Y. (1999). *Proc. Am. Soc. Clin. Oncol.*, **18,** 159.

Tan AR, Headlee D, Messmann R, Sausville EA, Arbuck SG, Murgo AJ, Melillo G, Zhai S, Figg WD, Swain SM and Senderowicz AM. (2002). *J. Clin. Oncol.*, **20,** 4074–4082.

Testa JR and Bellacosa A. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 10983–10985.

Thomas JP, Tutsch KD, Cleary JF, Bailey HH, Arzoomanian R, Alberti D, Simon K, Feierabend C, Binger K, Marnocha R, Dresen A and Wilding G. (2002). *Cancer Chemother. Pharmacol.*, **50,** 465–472.

Tsuchida E and Urano M. (1997). *Int. J. Radiat. Oncol. Biol. Phys.*, **39,** 1153–1161.

Usuda J, Saijo N, Fukuoka K, Fukumoto H, Kuh HJ, Nakamura T, Koh Y, Suzuki T, Koizumi F, Tamura T, Kato H and Nishio K. (2000). *Int. J. Cancer*, **85,** 275–280.

Walker DH. (1998). *Curr. Top. Microbiol. Immunol.*, **227,** 149–165.

Wang MB, Billings KR, Venkatesan N, Hall FL and Srivatsan ES. (1998). *Otolaryngol. Head Neck Surg.*, **119,** 593–599.

Wang C, Fu M, Mani S, Wadler S, Senderowicz AM and Pestell RG. (2001). *Front. Biosci.*, **6,** D610–D629.

Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA and O'Connor P. (1996). *J. Natl. Cancer Inst.*, **88,** 956–965.

Wang Q, Worland PJ, Clark JL, Carlson BA and Sausville EA. (1995). *Cell Growth Differ.*, **6,** 927–936.

Warbrick E, Lane DP, Glover DM and Cox LS. (1995). *Curr Biol*, **5,** 275–282.

Weinberg RA. (1996). *Cytokines Mol. Ther.*, **2,** 105–110.

Werner J, Kelsen D, Karpeh M, Inzeo D, Barazzuol J, Sugarman A and Schwartz GK. (1998). *Proceedings of the American Society of Clinical Oncology* Vol. 17, Los Angeles, CA.

Wilcken NR, Sarcevic B, Musgrove EA and Sutherland RL. (1996). *Cell Growth Differ.*, **7,** 65–74.

Wilson WH, Sorbara L, Figg WD, Mont EK, Sausville E, Warren KE, Balis FM, Bauer K, Raffeld M, Senderowicz AM and Monks A. (2000). *Clin. Cancer Res.*, **6,** 415–421.

Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Buschenfelde KH and Beach D. (1995). *Science*, **269,** 1281–1284.

Worland PJ, Kaur G, Stetler-Stevenson M, Sebers S, Sartor O and Sausville EA. (1993). *Biochem. Pharmacol.*, **46,** 1831–1840.

Wright J, Blatner GL and Cheson BD. (1998). *Oncology (Huntingt)*, **12** 1018, 1023–1024.

Yu JT, Foster RG and Dean DC. (2001). *Mol. Cell. Biol.*, **21,** 3325–3335.

Yu L, Orlandi L, Wang P, Orr M, Senderowicz AM, Sausville EA, Silvestrini Ra and O'Connor P. (1998). *J. Biol. Chem.*, **273,** 33455–33464.

Zaharevitz DW, Gussio R, Leost M, Senderowicz AM, Lahusen T, Kunick C, Meijer L and Sausville EA. (1999). *Cancer Res.*, **59,** 2566–2569.

Zhou Q, Stetler-Stevenson M and Steeg PS. (1997). *Oncogene*, **15,** 107–115.

Zhu G, Conner S, Zhou X, Shih C, Brooks HB, Considine E, Dempsey JA, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B and Watkins SA. (2003a). *Bioorg. Med. Chem. Lett.*, **13,** 1231–1255.

Zhu G, Conner SE, Zhou X, Shih C, Li T, Anderson BD, Brooks HB, Campbell RM, Considine E, Dempsey JA, Faul MM, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B and Watkins SA. (2003b). *J. Med. Chem.*, **46,** 2027–2030.